ON A PLATYPUS EMBRYO FROM THE INTRA-UTERINE EGG.

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(Plates ix.-xiii.)

Introduction.

The following paper is based on the examination of two embryos taken from the intra-uterine eggs of a Platypus. Beyond the facts that Monotremes are oviparous and the ovum is meroblastic the material collected by Caldwell in 1884 has afforded us very little information, and we have thought that a description of a Platypus embryo of this stage may not be unwelcome to zoologists. In this paper we necessarily confine ourselves to a description of the structure of the embryo lying before us. Next year, now that we know the exact breeding season of Platypus in certain convenient localities in New South Wales, we shall endeavour to obtain the stages intermediate between the earliest we now possess and the embryo described in this paper.

The female from the left uterus of which the two eggs were taken was shot on 1st October of this year. The general external characters of the egg have already been sufficiently accurately described.* The eggs were both exactly of the same size and spheroidal in shape. The egg shell is, as Caldwell described, of an opaque white colour and quite soft, presenting a general resemblance to the shell of a lizard's egg.

The eggs measured 18 mm. in their long and 13·5 mm. in their short diameter. They are thus somewhat larger than the eggs secured by Caldwell, who gives the measurements of the egg when

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* Caldwell, Phil. Trans. 1887, p. 473.

laid as 15 mm. by 12 mm. Three other females were shot on the same date, and these had obviously just laid their eggs, as evidenced by the emptiness and large size of the left uterus and by the presence and condition of corpora lutea in the ovary. Moreover, the mammary glands in all four females were of approximately the same size. The tubules were arranged in a fan-like fashion, radiating outwards from the, at this stage, very small bare area of the ventral abdominal wall, and measured 5 cm. in length.

From the size of these eggs as compared with Caldwell's, and from the condition of the other three females shot on the same date, we may reasonably conclude that they were just ready to be laid.

The only other recorded measurements besides Caldwell's of the size of the Platypus eggs when laid are contained in a paper by Geoffroy St. Hilaire published in 1829.* The eggs, nine in number, were found lying on a rough nest in a small burrow on the banks of the River Hawkesbury, N.S.W., and measured $1\frac{3}{8}$ inches (34 mm.) long by $\frac{6}{8}$ of an inch (19 mm.) broad. The eggs here described were probably not those of Platypus at all: as St. Hilaire himself afterwards pointed out, they could not on account of their size pass through the pelvis, and he then came to the conclusion that in Platypus the eggs must be hatched inside!

The eggs were opened immediately after the animal was shot and their contents preserved in picro-sulphuric acid. The embryos were stained with borax-carmine, imbedded in paraffin and cut into serial sections with the Cambridge microtome.

To our friend, Prof. J. T. Wilson, we are indebted not only for many valuable suggestions but for much kindly criticism during the course of our work, and we desire here to tender him our sincere thanks. We have also to thank Messrs. Shewen and Grant, assistants in the Physiological Laboratory, for much assistance in the preparation of the photo-micrograph accompanying this paper.

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* Ann. des Sc. Nat. T. xviii. p. 162.

General Description of the Embryo as seen in Surface View.

On opening the egg, the embryo was found lying on the surface of a thin-walled vesicle, with its long axis corresponding to the long diameter of the egg. It extended over the surface of the vesicle almost from pole to pole. The vesicle completely filled the interior of the shell. It contained a thin whitish transparent fluid of an albuminous nature which was precipitated in picro-sulphuric acid. Immediately below the wall of the vesicle there appeared a thin layer of yolk granules which was somewhat increased over a small area at the ant-embryonic pole. The embryo measured 19 mm. from the anterior end of the medullary plate to the extreme posterior end of the primitive streak. This hinder point of measurement is 1·5 mm. behind the blastopore. A photo-micrograph of the embryo from the dorsal side magnified $5\frac{4}{5}$ diameters is shown in Pl. IX. Outside the elongated and somewhat fiddle-shaped contour of the embryo is seen a lighter more transparent zone (Pl. IX. *am. a.*) corresponding to the amniotic area of other mammals. In the fresh condition no trace of a vascular area was visible, though in the hardened blastoderm developing vessels were indicated by a mottling both in and around the amniotic area. Immediately in front of the anterior end of the embryo there was to be seen a lighter area—the proamnion—(Pl. IX., *pra.*) into which the mesoderm had not yet extended. The antero-lateral portions of the embryo were almost entirely occupied by two sharply limited patches situated one on either side of the anterior region of the medullary plate, opposite the position of the future first and second cerebral vesicles. The outer contours of these head plates are posteriorly in line with the forward continuations of the outer borders of the proto-vertebral zones of mesoderm. The outer margins of these head plates mark the lateral limits of a very considerable mesodermal thickening in this region, and we may for convenience of description term them the head plates of mesoderm. Their relations to the general mesoderm will be described later.

The commencing separation of the embryo from the vesicle is indicated by the presence of a sulcus, the so-called head-fold, which passes back for a distance of approximately ·17 mm. below the anterior end of the medullary plate and thus separates the latter from the underlying proamnion. This sulcus we must suppose has arisen as in other forms by the forward growth of the anterior end of the medullary plate over this bilaminar portion of the blastoderm.

Lateral and tail folds are not yet developed.

The medullary plate is still flat with the exception that along its mesial line a definite groove (the "Rückenfurche") is developed. Medullary folds are absent throughout except in the region of the future fore-brain.

In the head region the three future cerebral vesicles are indicated by widenings of the medullary plate. The first is separated from the second by a well marked constriction, while a less marked constriction situated somewhat anterior to the posterior margins of the mesodermal head plates separates the second from the third.

The upgrowths of the medullary folds in the lateral regions of the fore-brain are very apparent in the photo-micrograph as two dark lines (Pl. IX., *md.f.*). The continuation of these lines across the front of the medullary plate is produced by the thickening and duplication of the medullary plate at the head fold (Pl. XI., figs. 15 and 16 *cp.*).

The medullary plate in the region of the hind-brain is especially characterised by the presence on each side of four oblong metamerically arranged opaque masses extending from the outer edges of the medullary plate to within a short distance of the mesial line. These, as will be shown later, are local thickenings of the medullary plate, and are therefore true neuromeres. The neuromeres are arranged in bilaterally symmetrical pairs, and adjacent ones are separated from each other by well marked transverse constrictions. The first pair (Pl. IX., *n.*^{1}.) are situated entirely in front of the auditory plates. They are narrow and transversely elongated. The second pair are not so distinct, and do not extend quite so far mesially as the first. The third pair are very distinct and somewhat broader than the first. They are directed slightly backwards. The fourth pair are much less distinct than the others, and in surface view are not so sharply limited from the medullary plate.

In the anterior region of the mid-brain, a pair of neuromeres (Pl. IX., N.) occur, but they are much less distinct than those of the hind-brain, appearing merely as local thickenings of the medullary plate in that region, without any accompanying constrictions.

Opposite the second and third neuromeres of the hind-brain there is on each side a somewhat triangular thickened patch of ectoderm—the auditory plate (Pl. IX., *aud.*). The mesoderm in the region of the auditory plates is very thin, hence they stand out very distinctly. The anterior margin of each plate is on a level with the front edge of the second neuromere; indeed the greater portion of the plate is situated opposite this neuromere. Each plate is roughly triangular in shape, with the base of the triangle adjoining the medullary plate, and with a deep bay in the middle of its posterior margin.

The hind-brain region narrows gradually posteriorly and passes into the medullary plate of the future spinal cord. The medullary plate widens out towards the posterior end of the embryo into a well marked sinus rhomboidalis which completely surrounds the primitive streak. The primitive streak is just visible in the photo-micrograph (Pl. IX., *pr. s.*) as a faint linear thickening enclosing a whitish axial line—the primitive groove—at the posterior end of the embryo. The anterior end of the primitive streak exhibits a distinct thickening, to one side of which the blastopore (Pl. IX., *bl.*) is situated. This thickening is continued forwards as the head process of the mesoderm which passes into the hinder end of the notochord. The notochord is very distinct in the photo-micrograph as the longitudinal line running along the middle of the medullary plate. At its anterior end it broadens out, and terminates about the middle of the future fore-brain.

The bilateral Anlagen of the heart are very noticeable in surface view (Pl. IX., *h.a.*) as two tubular-looking structures lying external to the auditory plates, and extending backwards from them along the outer edges of the forward extensions of the lateral zones of mesoderm, nearly up to the first somite. They are thus situated in greater part opposite the hind-brain region.

In the trunk region, on either side of and extending below the medullary plate there are seventeen pairs of somites: the first pair situated relatively far back with regard to the auditory plates, and separated by a considerable space from the last neuromere. The anterior somites are square in shape with their borders at right angles to the axial line. They become successively broader and shorter towards the posterior end of the embryo, where they are placed obliquely to its long axis.

On each side just external to the outer edges of the somites (with the exception of the first three) and between them and the lateral zone of mesoderm there occurs a narrow intermediate zone containing the Anlage of the Wolffian body (Pl. IX., *w.b.*). Beginning as a faint line opposite the fourth somite, it becomes more distinct opposite the seventh, and from thence backwards as far as the fifteenth somite it exhibits an irregular linear thickening. Over this posterior part of its course the Wolffian duct occurs as a distinct structure. Behind the fifteenth somite the intermediate cell mass extends backwards as a narrow strip somewhat beyond the last somite.

Caldwell* compared the Platypus embryo from an egg just laid to a chick of about 36 hrs., but beyond the number of somites being about the same in both, there is hardly any other point of comparison. In a chick of this age the medullary groove is closed right down to the sinus rhomboidalis, the head is covered by the amnion, the three cerebral vesicles and the optic vesicles are well developed, the heart is formed and already bent, the vascular area differentiated and the blood circulating; whereas in the Platypus embryo at about the time of laying, the medullary plate is practically flat, vascular area and amnion are non-existent, while the heart is represented by two lateral Anlagen at the periphery of the anterior extensions of the lateral plates of mesoderm.

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* Proc. Roy. Soc. N.S.W. Vol. xviii. 1884, p. 120.

In fact we are not acquainted with any embryo which reaches the dimensions mentioned above and is possessed of such a number (17 pairs) of somites and which yet remains, with the exception of a slight head fold, absolutely flat.

Selenka's* figure (fig. 1 Taf. xxi.) of a three days' Didelphys embryo does however present considerable points of resemblance to the Platypus embryo under consideration, though it is very much smaller (4·5 mm.) and possesses only fourteen somites.

In both embryos the medullary plates are practically flat, double heart Anlagen are present, and head fold formed. The appearance of the anterior end of the medullary plate of the three days' Opossum closely resembles that of the Platypus embryo; and further, in the region of the future mid-brain the same lateral mesodermal thickenings occur (*vide* Selenka's fig. 4, Taf. xx. *y.*) as we have described above, though in the Opossum they are not so marked as in our embryo. According to Selenka these mesodermal thickenings "gehören offenbar zur Urwirbelplatte des Kopfes."

In the Opossum embryo neither the Wolffian body nor the auditory plates are indicated, nor are there any neuromeres described. It however seems highly probable to us that the structures situated in the region of the hind-brain which Selenka regards as the anterior five somites are in reality not somites at all but true neuromeres. A comparison of his fig. 4, Taf. xx., which represents a slightly younger embryo, with the above-mentioned figure renders this view still more likely. In his figures the structures regarded as the anterior five somites extend inwards from the edges of the medullary plate to within a short distance of the mesial line, and in surface view appear related essentially as are the neuromeres in our embryo; while the remaining somites of the trunk, instead of ending on a level with the edges of the medullary plate, extend out beyond them.

Further, his transverse section (fig. 3, Taf. xxi.) through the region of the hind-brain and passing through one of these supposed somites shows no mesodermal differentiation which could

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* Studien über Entwick. der Thier. iv. Lief. i Abt.

give rise to the appearance seen in surface view. The mesoderm extends out as a continuous plate of uniform thickness beyond the edge of the medullary plate, whereas the medullary plate itself shows a very noticeable thickening as compared with that of the other sections figured.

The marked retardation in the formation of the medullary folds and in the folding off of the embryo is one of the most characteristic features of the embryo at this stage. This may be due, as suggested by our friend Prof. J. T. Wilson, to the mechanical effect of the rapid imbibition by the ovum of nutritive fluid secreted by the uterine glands.

The mature ovarian ovum, according to Caldwell,* measures only 2·5 to 3 mm. in diameter. After the entrance of the ovum into the Fallopian tube the shell membrane and proalbumen are added externally to the vitelline membrane, and at the same time the ovum is increasing in size by the absorption of fluid. The youngest stages in our possession are eggs in which segmentation has advanced to some extent; they measure 5 mm. in diameter, and possess a distinct and resistent shell membrane separated from the vitelline membrane by a thin layer of proalbumen. As development proceeds this layer of proalbumen is soon wholly absorbed, and in the eggs under consideration the blastodermic vesicle tightly distended with fluid fitted closely around the inner surface of the fully formed shell, the vitelline membrane being no longer recognisable. During the rapid imbibition of fluid by the blastodermic vesicle, and the consequent increase in size of the whole ovum, the wall of the vesicle including the embryonal area is closely pressed against the surrounding vitelline and shell membranes. It therefore seems reasonable to suppose that under such conditions, only those structural features of the embryo are produced which do not involve any upgrowths of the wall of the vesicle. Once the definite shell is fully formed around the egg and no possibility exists of its obtaining a further supply of maternal nutritive material, the normal development of bodily

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* Phil. Trans. 1887.

form by folding of the blastoderm is enabled to occur by the embryo making room for itself, so to speak, by the using up of the fluid contents of the vesicle.

Against the view here put forward, it may be urged that in certain other forms there is a similar rapid increase in size of the blastodermic vesicle by the absorption of fluid, and yet there is no retardation in development. In Didelphys, for example, the blastodermic vesicle, according to Selenka, increases in one day from a diameter of 6 mm. to one of 15 mm., and at the end of this time the embryo is folded off, the medullary groove is closed and the amniotic folds developed. All these processes can, however, easily occur on an expanding blastodermic vesicle lying naked in the cavity of the uterus and devoid of any such mechanical obstacle as would be presented by the presence of a resistent shell membrane.

Ectoderm.

The ectoderm forms a continuous covering for the whole of the blastodermic vesicle. It consists, except in the regions to be subsequently mentioned, of a single layer of polygonal cells. Over the greater portion of the embryonic area the cells are much flattened, while in the head region of the embryo and in the extra-embryonic region of the wall of the vesicle they appear cubical in section.

Medullary plate.—The medullary plate is, as already mentioned, still practically flat. Medullary folds are only present in the anterior region of the future fore-brain; their appearance in this region is probably to be associated with the very early appearance of the optic grooves. The plate consists of elongated cells, the nuclei of which are situated at different levels simulating the appearance of several layers of cells. The lateral portions of the plate are thickest, and are connected by a median much thinner portion which sends down a keel-shaped process in some parts to meet the notochord. Along the median portion of the plate there runs a distinct groove—the "Rückenfurche." Beginning as a shallow groove slightly behind the anterior end of the medullary plate, it gradually becomes deeper posteriorly, attaining its maximum depth just anterior to the 1st pair of somites, where it is distinctly V-shaped (fig. 7, *d. fr.*). Behind this point it gradually becomes shallower again until in the region of the 8th somite the medullary plate is almost flat, and much thinner than anteriorly (figs. 9 and 10, *md. p.*). Posterior to the somites the plate gradually increases in breadth to form the sinus rhomboidalis which invests the primitive streak. The extreme anterior end of the medullary plate is quite flat, destitute of a median groove, and separated from the underlying proamnion by the head fold. It therefore consists of two layers, a thicker upper and a thinner lower layer, separated from each other by a narrow space (figs. 1, 15 and 16).

Immediately behind the anterior end of the medullary plate its margins become upturned to form the medullary folds which are conspicuous in the photo-micrograph as the two dark lines on either side of the future fore-brain. A transverse section through the middle region of the fore-brain is shown in fig. 2. In the middle of the section is a well-marked groove (*d. fr.*) separated by elevations of the medullary plate from two lateral grooves (*op. gr.*) bounded externally by the medullary folds which curve slightly inwards above the grooves. The median groove will form the future first cerebral vesicle, while the lateral grooves we regard as the Anlagen of the future optic vesicles. Heape* figures a section very similar to our fig. 2 through the optic grooves of the Mole (Stage F.), and comments on their very early appearance, while the medullary groove is still widely open. Posteriorly each optic groove deepens, becomes somewhat V-shaped in section, and at the same time its floor thickens, eventually forming a hollow outgrowth which arises from the bottom of the groove and proceeds outwards and downwards (fig. 3). An appearance similar to this has not been described, so far as we are aware, for any other embryo, and from the evidence at our disposal it would be rash to speculate too far as to its probable significance. The whole

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* Quart. Jour. Micros. Science, Vol. xxvii. 1887, fig. 16, Pl. XI.

appearance somewhat suggests a precocious formation of the secondary optic cup, but whether this is so or not can only be settled by the examination of older stages. We are convinced, however, from the examination of serial transverse sections through the region in question, that the appearance is not caused by any artificial folding. Longitudinal sections of our second embryo also exhibit a series of appearances contradicting such an interpretation.

Neuromeres.—As already mentioned four distinct pairs of neuromeres are present in the hind-brain and a less marked pair in the anterior region of the mid-brain. The mid-brain neuromeres are not so distinctly marked off from the medullary plate as those of the hind-brain. It is, however, obvious in longitudinal section as a distinct local thickening of the medullary plate (fig. 16, N.) corresponding to the region marked N in the photo-micrograph.

In longitudinal section the neuromeres exhibit the characteristic arc-shaped form originally described by Orr* in the Lizard (Anolis), and afterwards by McClure† in the Chick, in Amblystoma and Anolis, and by Waters‡ in the Cod.

The neuromeres in the hind-brain of Platypus exhibit very closely the characters described by Orr for those of the hind-brain of the Lizard. It must, however, be remembered that in the embryo Lizard, to which Orr's description applies, the medullary canal is closed, while in the Platypus embryo the medullary plate is still flat, so that the outer surface of the medullary canal of the Lizard corresponds to the undersurface of the medullary plate in our embryo, and his inner surface to our upper surface.

Each neuromere as seen in longitudinal section (fig. 16, N.) is formed by an arc-shaped bulging on the under side of the medullary plate. Adjacent neuromeres are separated from each other by well-marked constrictions on the under side of the

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* Orr, Journ. Morphology, Vol. i, 1887, Pl. XII. fig. 5.

† McClure, Journ. Morphology, Vol iv. p. 35-56.

‡ Waters, Quart. Journ. Micros. Science, Vol. xxxiii. p. 457-472.

medullary plate, while on the upper side, opposite the constrictions, there are slight transverse ridges.

The elongated cells of which each neuromere is composed are distinct from those of its neighbours. The cells are arranged radially from the upper surface of the neuromere, and their nuclei are slightly more numerous just below that surface (fig. 18). In the hind-brain of the lizard, on the other hand, according to Orr, the nuclei are more numerous towards the outer surface of the neuromere, *i.e.*, towards the undersurface in the medullary plate stage.

In transverse sections the first neuromere of the hind-brain appears as a thickening of the medullary plate with two bulgings on its ventral side—a smaller one situated near the middle of each half of the medullary plate, and a larger one at the outer edge of the plate [fig. 19 (2)]. The outer bulging projects considerably beyond the lateral margins of the medullary plate in the interneuromeric region.

The second neuromere [fig. 19 (4)] is less marked than the first, but also possesses mesial and lateral bulgings. The third neuromere [fig. 19 (6)] is on surface view the most distinct of the four. It possesses a single large bulging at its outer edge. The fourth neuromere [fig. 19 (8)] is the least distinct; it possesses, like the first and second, two enlargements of which the lateral one is the larger (fig. 17). Immediately behind the fourth pair of neuromeres of the hind-brain the medullary plate is thickened, but the thickenings are not limited behind by constrictions, and for the present we leave it an open question whether these are to be regarded as a fifth pair of neuromeres or not.

Neuromeres in the fore-brain were not observed. All that we can definitely say at present, then, is that in the head region of the Platypus embryo of this stage a single pair of neuromeres exist in the mid-brain and four distinct pairs in the hind-brain. As Locy* has observed in *Squalus acanthias*, and *Amblystoma*, so in Platypus the neuromeric segmentation appears very early, indeed

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* Anat. Anz. ix. Bd. p. 393-415.

before the formation of the medullary folds. The same observer has also insisted on the fact that the neuromeric segmentation is primitively ectodermic and entirely independent of any mesodermic segmentation—a view with which the conditions in Platypus are in complete agreement.

Auditory plates: The auditory plates are situated laterally to the neuromeric region of the hind-brain, their anterior edges being on a level with those of the second pair of neuromeres. Each consists of a thickening of the ectoderm which is distinctly grooved longitudinally (fig. 6, *aud.*). The appearance of the auditory plates in Platypus while the medullary plate is still flat is noteworthy.

Cranial ganglia are not yet developed. However, in the interval between the third and fourth pairs of neuromeres and opposite the posterior portions of the auditory plates there occurs on each side a distinct downgrowth of the ectoderm just external to the outer edge of the medullary plate. This downgrowth is similar to the "Zwischenrinne" described by His[*] in 1879. Beard,[†] Rabl,[‡] Chiurugi,[§] and others have observed a similar structure, but do not regard it as concerned with the development of the cranial ganglia, Goronowitsch,[||] indeed, being of the opinion that it is an artificial production of the paraffin bath.

There remains to be noticed a longitudinal strand of cells on each side lying close beneath the ectoderm just external to the edge of the medullary plate and passing slightly inwards below its outer margin (figs. 8 and 9, *le.*). The strands are found in the trunk region of the embryo extending from the first pair of somites anteriorly to about the fourteenth pair posteriorly. They are much larger and more distinct anteriorly. Whether these strands are ectodermal in their origin and ganglionic in their significance

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[*] His, Untersuchungen über die erste Anlage des Wirbelthierleibes—Leipzig.

[†] Beard, Quart. Journ. Micros. Sc. Vol. xxix.

[‡] Rabl, Morphol. Jahrb. Bd. xv.

[§] Chiurugi, Arch. Ital. de Biologie, Bd. xv.

[||] Goronowitsch, Morph. Jahrb. Bd. xx. Heft 2, p. 201.

could not with certainty be determined at this stage. They exhibit no cellular connection with their surroundings and may possibly represent the detached neural crest ("Zwischenstrang") of which no other representative is present.

Entoderm.

The entoderm of the embryonic area presents no very special features. It is a single layer of flattened cells, the nuclei of which are fairly close. Laterally the cells become more elongated and the nuclei consequently are further apart. Here and there in the embryonic area these flattened entodermal cells are interspersed with large cells distended by the presence in their interior of several yolk spheres (*cf.* fig. 22, *vit. ent.*). Further out these yolk-containing cells become more numerous and eventually form the entire inner lining of the blastodermic vesicle. Their structure is described later in connection with that of the vesicle. The only differentiation of the embryonic entoderm is found in the region of the future pharynx. The cells lying just internal to the amnio-cardial vesicles have assumed a cubical shape, and form a narrow thickened band on each side extending back to the region of the heart Anlagen (fig. 5, *ent. ph.*). These two bands represent the pharyngeal entoderm of this region.

Notochord: The notochord in surface view is seen to terminate about the middle of the future fore-brain, and sections through this region show that the notochord is here represented by a thickening of the entoderm forming a median band with which the mesoderm is continuous laterally. Many of the cells in this anterior region of the notochord exhibit mitotic division (fig. 14, *nch.*). This median thickening as traced back becomes more marked and more sharply limited laterally though it is not yet distinct from the entoderm. It is in contact above and for some distance behind this point with the keel-shaped process of the medullary plate. Then just posterior to this the notochord becomes distinct as a small rounded mass closely connected with the entoderm below and in contact with the keel-shaped process of the medullary plate above (fig. 4). Then, from about the middle region of the mid-brain up to a short distance in front of the first somite, the notochord lies below the keel of the medullary plate and is connected with the entoderm by a thin cellular filament (fig. 5). Somewhat anterior to the first somite the notochord becomes entirely free from the entoderm, and continues in this condition to its posterior end where it passes into the head process of the primitive streak. The notochord increases in size somewhat after becoming entirely free from the entoderm. It is then distinctly rod-shaped, while anteriorly it is somewhat oval in section.

The notochord is relatively of very small size in Platypus. Heape* has also noticed a similar condition in the Mole, and he regards it as due to the very early appearance of the nervous system.

Mesoderm.

The mesoderm is at this stage established as two lateral wings distinct from the ectoderm and entoderm except at certain regions in the axial line, viz.:—At the anterior flattened-out end of the notochord, in front of the blastopore in the region of the head process, and behind the blastopore in the region of the primitive streak. As already mentioned in the description of the surface view of the embryo, the mesoderm lying on either side of the anterior end of the embryo forms two sharply limited plates to which we have given the name of mesodermal head plates as distinguished from the mesoderm of the rest of the body.

The head plates of mesoderm (Pl. IX., *h.p. mes.*) are lateral thickenings on either side of the future fore- and mid-brains, and show no signs of segmentation. Their outer contours are directly continuous with the forward continuation of the outer boundaries of the protovertebral zones of mesoderm. Their very distinct posterior boundary is not due to the entire disappearance of mesoderm at this point, but to a very marked thinning of the same.

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* Quart. Journ. Micros. Science, Vol. xxvii. 1887, p. 139.

A transverse section through the middle region of the head plate is shown in fig. 4. Here, below the medullary plate, the mesoderm exists in the form of scattered stellate cells, while laterally to the outer edges of the medullary plate the cells are more numerous and more closely packed, especially immediately below the ectoderm and at the outer rim of the head plate. This thickened rim marks the outer contour of each plate as seen in surface view. Beyond this rim the mesoderm is divided by the development of a cœlom into two layers, an upper thin layer of flattened somatic mesoderm cells and a lower thicker layer of somewhat cubical splanchnic mesoderm cells. The narrow cœlomic spaces thus enclosed when traced posteriorly are found to be the most anterior parts of the body cavity, and for them we adopt Minot's* term amnio-cardial vesicles.

The amnio-cardial vesicles extend forwards as two horns beyond the anterior end of the embryo. They converge towards the median line without, however, uniting, and practically limit a small area (the proamnion) in which mesoderm is absent. The proamnion is of very small extent and lies immediately in front of and below the anterior end of the medullary plate (figs. 1 and 15, *pra.*). Behind the posterior limits of the head plates the mesoderm becomes reduced to a very thin layer, and is absent altogether over a limited area just anterior to the outermost portion of each auditory plate (fig. 5), and here ectoderm and entoderm come into contact as in the proamnial region. The thin areas of mesoderm behind the head plates are very obvious in the photo-micrograph as the lighter areas in the middle of which the auditory plates are situated. These thin areas are wholly confined to the forward continuations of the protovertebral zones of mesoderm. Externally to the thin areas are the forward extensions of the lateral trunk zones of mesoderm, along the outer edges of which are situated the symmetrical heart Anlagen.

The mesoderm in the hind-brain region is entirely destitute of segmentation. It consists, below the medullary plate, of scattered

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* Human Embyology, 1892, p. 198.

stellate cells which become somewhat more compact below the ectoderm and immediately external to the edges of the medullary plate. The outer margin of this more compact portion of the mesoderm corresponds to the outer limit of the forward prolongation of the protovertebral zone of mesoderm, and is visible in the photo-micrograph as the dark contour bounding this area. Beyond this line the mesoderm is split into the somatic and splanchnic layers. The somatic layer is composed of a single layer of cells and is closely applied below the ectoderm; the splanchnic layer is thicker, especially where it is inbulged over the heart endothelium (figs. 5 and 6, *spl.*). The two layers unite again into a single layer a little way external to the lateral heart Anlage. The lateral extension of the unsplit mesoderm beyond the heart Anlage is, however, very small, so that the lateral extent of the cœlom practically corresponds in this region with the lateral extent of the mesoderm. From this point backwards the mesoderm gradually extends more and more outwards until it reaches its maximum extension opposite the posterior end of the embryo.

Behind the heart Anlagen proper the somatic layer of mesoderm becomes very much thicker than the splanchnic (figs. 7, 8, 10 and 12), and it continues in this condition to the posterior end of the embryo. At the same time the two layers become more closely applied to each other and the cœlom is reduced to a narrow cleft. Just over the venous trunks leading to the heart Anlagen the two layers are unsplit, thus dividing the cœlom into a more mesial and a more lateral portion.

The splitting of the more mesially situated part of the lateral mesoderm becomes more indistinct posteriorly, so that opposite the anterior somites the mesial part of the ventral cœlom is largely obliterated and there exists external to the somites a mass of unsplit mesoderm (fig. 8).

The par-axial mesoderm immediately in front of the first pair of somites, though not transversely limited in front as a distinct segment, has essentially the same appearance in section as that of the first somite. It forms on each side a distinct and compact plate lying close below the ectoderm immediately external to the edge of the medullary plate. Beneath it there exist looser stellate cells which are continuous with it at both ends (fig. 7, *mes. ax.*).

Mesodermic somites: The appearance of the seventeen pairs of somites as seen in surface view has already been described. With the exception of the first three and the last three the somites are practically identical. In transverse sections (figs. 8 and 9, *m.s.*) they present an oblong form compressed dorso-ventrally and extend some distance beneath the medullary plate. They possess in their whole breadth very distinct myotomic cavities bounded by dorsal and ventral walls composed of somewhat stellate cells. The cavity is sometimes interrupted by strands of cells passing between the two walls.

The ventral walls of the first three pairs of somites have become converted into stellate mesenchyme cells, and in the first at least the myotomic cavity is no longer distinguishable (fig. 7). Their dorsal walls form a somewhat arched plate of closely compacted cells.

The last three pairs of somites do not possess well marked cavities. They consist of about two layers of cells connected by cellular bridges (fig. 10, *m.s.*).

Lateral trunk mesoderm and ventral cœlom: The lateral mesoderm appears directly continuous with the first three somites, without any intermediate cell mass, while from the 4th onwards a distinct intermediate cell mass is present between the two (figs. 8 and 9).

Except opposite the posterior somites the cleavage of the lateral mesoderm does not extend right up to the somites, the cœlom only appearing some distance out. The splanchnic layer is only one cell thick, while the main portion of the mesoderm continues out as a thick somatic layer. This gradually thins as it passes out, becomes reduced to a single layer of cells, and ultimately fuses with the splanchnic layer to constitute a single mesodermal layer marginally.

The dark area in the photo-micrograph just external to the somites is the optical expression of this thick somatic layer of mesoderm. The outer limit of the dark area marks the place where the latter becomes reduced to a single layer.

The ventral cœlom is, in the region of the 1st somite and just anterior to it, coextensive with the thickened portion of the somatic mesoderm seen in surface view, while posteriorly it extends out beyond the point where the latter becomes thin. Further back still the cœlom gradually becomes reduced in extent until in the region of the 9th to the 13th somites the mesoderm is no longer split (fig. 9, *mes.*).

Opposite the 14th or 15th somites the mesoderm again becomes split, the cœlom extending close up to the intermediate cell mass (fig. 10).

Behind the somites the protovertebral zones of mesoderm are directly continuous with the lateral plates, while the splitting of the mesoderm does not occur until some distance out (fig. 12).

The mesoderm continues some distance beyond the hinder end of the primitive streak, and here the cœlomic cavities gradually extend inwards towards the mesial line and fuse with each other, so that the cœlom forms a continuous space. In this region the tail fold of the amnion will probably be developed.

Primitive streak : In surface view the notochord is seen to become gradually thicker at its posterior end and to terminate finally in a distinct longitudinal thickening situated about the middle of the sinus rhomboidalis. The continuation forwards of this enlargement to join the notochord is the head process of the primitive streak, while behind it is the primitive streak itself, just visible in the photo-micrograph as a whitish line.

Sections through the primitive streak show that mesodermal cells are being rapidly proliferated off from the ectoderm forming the floor of the primitive groove along its whole extent, and that the lateral wings of mesoderm are directly continuous with this axial streak of cells (figs. 12, 13, and 24). At the anterior end of the primitive groove ectoderm, mesoderm and entoderm are fused together in the axial line (figs. 23 and 24) and form the enlargement already referred to which projects as an elongated eminence composed of rounded cells at the anterior end of the primitive groove (figs. 11 and 23). In the hollow at one side of this eminence the blastopore (*bl.*) is situated. The blastopore leads into the blastoporic canal which runs forwards in the head process for a distance of 16 mm., and opens by a lateral opening into the cavity of the blastodermic vesicle (fig. 21, *bl. op.*). The inner opening of the blastoporic canal appears as a break in the entoderm. The walls of the canal are wholly composed of mesoderm. The lumen of the blastoporic canal is not a single one, but is divided up by bridges of cells into two or three smaller canals (fig. 22, *bl. c.*). A similar duplication of the canal has been observed by Kölliker* in the rabbit, by Bonnet† in the sheep, by Zumstein‡ in the chick, and by Spee§ in the guinea-pig; hence the latter observer regards it as probably of general occurrence in the formation of the chorda in mammals.

The head process runs forwards from the front end of the primitive streak, distinct from the ectoderm though closely applied below it. Laterally it is continuous at intervals with the mesoderm, while below it exhibits traces of cellular connection with the entoderm. This connection with the entoderm is interesting in view of the observations of Carius|| that the head process is free at first and only subsequently unites with the entoderm. Just anteriorly to the inner opening of the blastoporic canal the head process is connected with the ectoderm by a narrow median strand exhibiting a similar appearance to that shown by Graf Spee¶ for the guinea-pig. Two small lumina at this stage not continuous with the blastoporic canal occur in the head process just anterior to the inner opening of the canal (fig. 20, *nch. c.*),

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* Sitzungsber. Phys-med. Gesellschaft in Würzburg. 1883.

† His's Archiv. 1884.

‡ Mesoderm. 8vo, Bern, 1887.

§ Anat. Anz. iii. Jahrg. 1888.

|| 8vo. Marburg, 1888.

¶ Anat. Anz. 1888, p. 319 (et fig. 2).

while a short distance further forward a single distinct lumen occurs, but it is confined to one section. Apparently we have here to do with the last traces of the notochordal canal.

The head process diminishes in thickness anteriorly and finally passes into the posterior end of the notochord.

Wolffian duct and body: The appearance of the Anlagen of the Wolffian duct and body has already been described in surface view. In sections of its anterior region from the 4th to the 7th somites the united Anlagen of the Wolffian duct and body appear as a solid cord of cells projecting from the intermediate cell mass. The greater part of the cord lies free between the outer edges of the somites and the lateral mesoderm, while its dorsal surface approaches within a short distance of the ectoderm (fig. 8, *w. b.*). As the cord is traced backwards it is found to become gradually constricted in its middle region, while its dorsal portion broadens out, until it becomes somewhat dumb-bell-shaped in form. Its basal portion is now very distinctly connected with the lateral mesoderm on its outer side. On its inner side, however, the connection with the somites is not now so well marked, and in places this connection is completely lost. At about the level of the 6th somite the constriction of the middle region of the cord is much more marked, and it here consists of a ventral larger rounded mass connected by a narrow isthmus with a dorsal much thinner flattened band. The upper portion is the Anlage of the Wolffian duct, while the lower is the Anlage of the Wolffian tubules. Then, by the gradual disappearance of the connecting isthmus the mass comes to consist of a dorsal band-like Wolffian duct Anlage (fig. 25, *w.a.*), united at its mid-region to the underlying Anlage of the tubules (*w.t.*). Finally, opposite the 7th somite the narrow connection between the duct Anlage and the Anlage of the tubules is lost altogether, and the two become separate (fig. 26).

The outer edges of the duct Anlage lie close below the ectoderm which, just over the duct, is very thin and delicate, and with very few nuclei as compared with the rest of the ectoderm. Very often it is broken in the process of section cutting—a feature Martin* has also met with in the case of the rabbit (*cf.* his fig. 7 A-D. Taf. vii. in this respect with our figs. 25 and 26).

The Wolffian duct Anlage is now distinct not only from the somites and lateral mesoderm, but also from the Anlage of the tubules. However, it does again become connected with the tubule Anlage, and also with the lateral mesoderm at its outer edge, over a very short distance. The Anlage of the tubules consists of a somewhat rounded mass in transverse section, with its cells arranged in a radial manner round its somewhat clearer centre. In this in some sections a distinct lumen occurs (fig. 26, *w.t.*) The tubule Anlage is now only distinctly connected with the lateral mesoderm, the connection with the somites being lost more or less completely. Some sections indeed (fig. 26) show the tubule Anlage as an isolated rounded mass, below which passes a thin layer of loose cells of the intermediate cell mass.

The duct varies somewhat in width in different sections, and this gives rise to the irregular linear thickening previously mentioned as seen in surface view. As it is traced to its distal end the duct is found to be become gradually reduced to a thin flat plate somewhat thicker in the middle and thinning off laterally, and separated by a very small interval from the overlying thin area of the ectoderm. At the same time the Anlage of the tubule becomes reduced in size and its differentiation from the rest of the intermediate cell mass largely disappears, though it is still distinguishable as a compact mass of rounded cells forming a projection from the loose cells of the mass.

In its posterior region the outer edges of the now very thin Wolffian duct curve slightly upwards towards the ectoderm, and thus the entire Anlage has here a somewhat arc-shaped appearance. The duct is here of considerable width, though not more than two cells in thickness in its middle region. Finally, with the upturned edges of the Anlage, the ectoderm becomes continuous by means of fine but very distinct strands (fig. 27, *w.d.*), and there is thus

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* Archiv f. Anat. 1888.

enclosed between the two a small space. Behind the point where the connection of the edges of the Wolffian duct with the ectoderm is first seen, the duct rapidly becomes reduced in size and approaches closer to the ectoderm (fig. 28, *w.d*).

Finally it is reduced to a single cell, which passes directly over into the ectoderm (fig. 29, *w.d.*).

From these observed facts we are inclined to believe that the Wolffian duct in Platypus has an ectodermal origin. We cannot assert this dogmatically from the examination of one stage; yet the balance of evidence is in favour of this view, and indeed from the facts at our disposal it is the only view we can put forward.

The duct certainly does not grow backwards by proliferation from its posterior end as Martin states to be the case in the rabbit, for as opposed to the condition in that animal, where according to Martin the Wolffian duct at its extreme posterior end is thicker than just anterior to that point, in Platypus the duct gradually becomes thinner posteriorly, and as we have described, passes directly over into the ectoderm. Nor can the duct grow backwards by the addition of cells from the mesoderm, for as we have shown the Wolffian duct is quite distinct posteriorly from the Anlage of the tubules and from the adjacent mesoderm. We are therefore inclined to believe that the Wolffian duct in Platypus grows backwards by separation or delamination of cells from the ectoderm.

Just as the differentiation of the Anlage of the Wolffian duct from the ectoderm is lost as it is traced posteriorly, so the differentiation of the Anlage of the tubules from the intermediate cell mass is also lost. The Anlage of the tubules can, however, be traced behind the termination of the Wolffian duct as a narrow strand of rounded cells readily distinguishable from the looser branching cells of the rest of the intermediate cell mass. The relations of the Anlage of the tubules to the intermediate cell mass in Platypus is thus essentially the same as Martin has described for the rabbit.

In its topographical relations the Wolffian duct in Platypus agrees with the conditions described by Meyer* in man, where according to him the proximal part of the duct leads back from the mesoderm while its distal portion is connected with the ectoderm. Both Meyer and Martin agree in describing the proximal part of the duct as mesodermal in origin, but as to this we are not in a position to speak with certainty. However, in Platypus the proximal part of the united Anlagen of the Wolffian duct and tubules is related essentially as Martin describes for the corresponding portion in the rabbit, and it might well be that as in that animal the proximal portion of the Wolffian duct Anlage arises from the intermediate cell mass in common with the Anlagen of the Wolffian tubules.

Vascular System.

Heart Anlagen: The symmetrically placed heart Anlagen have already been described in surface view as situated in greater part opposite the hind-brain region. Sections, however, show that their anterior ends extend somewhat beyond the posterior limits of the head plates of mesoblast, and we may therefore look upon these head plates as the regions in which the future aortic arches will be developed.

The heart Anlagen at this stage in Platypus are essentially similar to those of a rabbit of about nine days.

As the amnio-cardial vesicles are traced from their anterior ends backwards, they gradually increase in lateral extent, and at the same time towards the posterior limits of the head plates the thick splanchnic layer of mesoderm separates from the entoderm. In the space thus formed on each side, rounded vasifactive cells appear. Posteriorly these vasifactive cells have formed the vascular endothelium of the anterior cardiac region, and this lies in the gutter-like groove—open towards the entoderm—formed by the inbulging of the thick splanchnic mesoderm into the amnio-cardial cœlom. Each heart Anlage consists anteriorly of several

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* Arch. f. Mikr. Anat. Bd. 36, 1890.

(2-3) endothelial tubes (fig. 5, *ht. end*) which about the middle region of the Anlage unite into a single tube (fig. 6, *ht. end.*). Traces of a septum are, however, still present in the single tube, showing that it has arisen, as Rabl* has observed, by the fusion of at least two smaller ones. On the ventral wall of the single endothelial tube there is a distinct cell mass projecting into the cavity of the same (fig. 6): it apparently represents the ventral part of the septum above mentioned. The endothelial wall is separated by a considerable space from the (splanchnic) mesodermal wall of the heart Anlage. In the middle region of the Anlage the latter exists in the shape of a semi-tubular canal open ventrally (fig. 6, *spl.*), while both in front and behind the groove becomes shallower and more closely applied to the somatic mesoderm.

Posteriorly, at the same time the endothelial tube is reduced in size and is continued backwards as the Anlage of the sinus venosus and omphalo-meseraic vein, and with this other endothelial tubes unite. Near its posterior end each venous Anlage consists of one or two small vessels which disappear finally just anterior to the first somite.

Endothelial vessels have already begun to appear in other parts of the embryonic region, *e.g.*, in the mesodermal head plate and especially where that underlies the medullary plate (figs. 3, 4, 5 and 6). These are not yet connected with the anterior prolongations of the heart Anlage, nor do they appear to contain blood corpuscles. It is worthy of note that the endothelial vessels may also occur in the somatic mesoderm, between it and the ectoderm. Bonnet† has also observed vessels in the somatic mesoderm in the sheep, but according to him they soon disappear. As already mentioned, a vascular area was not visible in the fresh condition, but sections reveal the presence of vasifactive cells and actual vessels in the extra-embryonic region (figs. 10, 12, 30, *vas. c.*, *b.v.*).

Both the vessels and the vasifactive cells become more numerous opposite the posterior end of the embryo. The vessels exist in

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* Morph. Jahrb. Bd. xv. p. 226.

† His's Archiv. 1889, p. 56.

the form of endothelial tubes which may enclose a number of vasifactive cells.

The vasifactive cells constituting blood islands occur in great numbers opposite the posterior region of the embryo between the more compact superficial layer of mesoderm and the entoderm (figs. 12, 30, *vas. c.*). In the mesial portion of this region the vasifactive cells appear to be differentiating to form vessels, while further out they occur in larger or smaller undifferentiated blood islands. The vasifactive cells possess each a large rounded nucleus with a very thin surrounding layer of protoplasm (fig. 30, *vas. c.*).

Structure of Blastodermic Vesicle.

The oval vesicle on which the embryo lies is comparable at this stage to a typical mammalian blastodermic vesicle, and forms in some respects a striking connecting link between the conditions obtaining in the Sauropsida and in the Placental Mammals.

The for the most part flattened ectoderm cells of the embryonic area pass into the more cubical cells forming the outer layer of the wall of the vesicle. Both ectoderm and entoderm form perfectly continuous layers all round the vesicle (fig. 32).

The vesicle, as already described, contained a thin albuminous fluid, while below its thin wall there existed a layer of yolk spheres. Sections and preparations of the wall of the vesicle mounted whole show that these yolk spheres are all intracellular. They are contained in large cells—vitelline entoderm cells—which, as has been already stated, are sparsely present among the flattened entoderm cells of the embryonic area, and immediately outside this are more abundant; while throughout the rest of the non-embryonic portion of the vesicle they constitute the entire inner entodermic lining of the latter.

The vitelline entoderm cells are of great size and are almost entirely occupied by large yolk spheres (figs. 30-33, *vit. ent.*). Each cell contains a large nucleus rendered somewhat irregular by internal compression by the yolk spheres. The nucleus is generally situated on the side of the cell next the ectoderm (fig.

33). The greater part of the chromatin of the nucleus is contracted into a star-shaped mass in the centre, while smaller particles of chromatin occur sparsely around this.

The mesoderm extends round from a quarter to a half of the circumference of the vesicle in the posterior region of the embryo. The lateral extension of the mesoderm diminishes gradually as one proceeds forwards, so that in the region of the heart Anlagen it extends only a short distance laterally to them, while in the region of the head plates of mesoderm the amnio-cardial vesicles form its outermost limit. In front of the embryo beyond the point where the amnio-cardial vesicles converge to limit the proamnion, mesoderm is entirely absent.

Beyond the cœlom there extends out a layer of flattened mesodermal cells between which and the vitelline entoderm is a layer of numerous rounded vasifactive cells (fig. 30, *vas. c.*). Further out these two mesodermal layers are continued into a layer of somewhat spindle-shaped cells with large rounded nuclei which forms the outermost portion of the extra-embryonic mesoderm (fig. 31, *mes.*). It is from the relatively very early great lateral extension of the mesoderm and from the presence of a very distinct yolk-containing entoderm that we regard the vesicle of the Platypus embryo of this stage as transitional between the yolk sac of Sauropsida and the typical mammalian blastodermic vesicle.

In the Sauropsida it is only after most of the yolk has been absorbed that the yolk sac is completely lined by discrete entodermal cells; in the higher mammalia, on the other hand, in the absence of yolk, the entoderm—the homologue of the yolk mass of Sauropsida—is very early able to completely enclose the cavity of the blastodermic vesicle—the homologue of the yolk sac cavity of Sauropsida. The ovarian ovum of the Platypus is as is well known a typical yolk-laden egg, yet at this stage the embryo, instead of overlying a mass of unsegmented yolk, lies on the surface of a two-layered vesicle containing fluid, which is only distinguishable from a typical mammalian blastodermic vesicle through the fact that instead of having a yolk-free entoderm, it possesses an entoderm composed of large yolk-containing cells.

EXPLANATION OF PLATES.

Reference Letters.

am. a. Amniotic area. *amc. v.* Amnio-cardial vesicle. *aud.* Auditory plate. *bl.* Blastopore. *bl. c.* Blastoporic canal. *bl.op.* Internal opening of blastoporic canal. *b.v.* Blood vessels *cœ.* Cœlom. *d. fr.* Median sulcus of medullary plate ("Rückenfurche"). *ect.* Ectoderm. *ent.* Entoderm. *ent ph.* Pharyngeal entoderm. *h.a.* Heart Anlage. *h. pr.* Head process of primitive streak. *hp. mes.* Head plate of mesoblast. *ht end.* Heart endothelium. *le.* Longitudinal strand of cells lying just external to the edges of medullary plate. *m.s.* Somite. *m.s.c.* Cavity of somite. *md. f.* Medullary fold. *md. p.* Medullary plate. *mes.* Mesoderm. *mes. ax.* Paraxial mesoderm in front of 1st somite. *mes. l.* Lateral mesoderm. *N.* Neuromere of mid-brain. *n'-n.*^{iv} Neuromeres of hind-brain. *nch.* Notochord. *nch.c.* Remains of notochordal canal. *op. gr.* Optic groove. *pra.* Proamnion. *pr. g.* Primitive groove. *pr. s.* Primitive streak. *som.* Somatic mesoderm. *spl.* Splanchnic mesoderm. *vas. c.* Vasifactive cells. *vit. ent.* Vitelline entoderm. *w. b.* Anlage of Wolffian body. *w. d.* Anlage of Wolffian duct. *w. t.* Anlage of Wolffian tubules.

Plate IX.

Photo-micrograph of Platypus embryo from the egg just ready to be laid. From the dorsal surface. (× $5\frac{4}{5}$.)

Plates X.-XIII.

The positions of the sections from which figs. 1-13 are drawn are indicated in the photo-micrograph by corresponding numbers.

All the drawings were made with a Zeiss' camera lucida.

Plate X.

Fig. 1.—Transverse section through the anterior end of the medullary plate, showing its separation from the vesicle, the proamnion (*pra.*) underlying it and the amnio-cardial vesicles (*amc. v.*) laterally. (× 70.)

Fig. 2.—Transverse section of the middle region of the future fore-brain passing through the commencement of the optic groove (*op. gr.*). The median sulcus (*d. fr.*) will form the future medullary groove proper. (× 70.)

Fig. 3.—Transverse section slightly posterior to fig. 2 showing the hollow outgrowth from the floor of the optic groove. (× 70.)

Fig. 4.—Transverse section passing through about the middle region of the head plate of mesoderm (*hp. mes.*). The amnio-cardial vesicles (*amc. c. v.*) form its outer limit. (× 70.)

Fig. 5.—Transverse section between the posterior limit of the head plate of mesoderm and the auditory plate. The section passes through the anterior end of the lateral heart Anlage—the endothelium (*ht. end.*) of which here consists of two tubes. Mesially to the heart Anlage the pharyngeal entoderm (*ent. ph.*) is visible. Mesially to the latter the mesoderm is interrupted over a small area. (× 70.)

Fig. 6.—Transverse section through the middle of the lateral heart Anlage. The endothelium here forms a single tube, though traces of a septum are still visible in it. The (splanchnic) mesodermal wall (*spl.*) of the heart has here a semitubular shape. The section also passes through the 3rd neuromere of the hind-brain (n'''.) apparent as a thickening of the medullary plate and through the grooved auditory plate (*aud.*). (× 70.)

Fig. 7.—Transverse section through the region immediately in front of the 1st somite. The paraxial mesoderm (*mes. ax.*) here exists in the form of an arched plate, below which are numerous stellate cells. The lateral mesoderm is not completely split, the cœlom being represented by several interrupted spaces. The median sulcus (Rückenfurche) (*d. fr.*) of the medullary plate is here very marked and the notochord is now quite free from the entoderm. (× 70.)

Plate XI.

Fig. 8.—Transverse section through the 6th somite. The somite somewhat oblong in section, is seen to extend mesially below the medullary plate and to possess a very distinct cavity (*m. s. c.*). The somite is separated from the lateral mesoderm by the Anlage of the Wolffian body (*w. b.*) Ventrally the latter is distinctly connected with the lateral mesoderm, while the connection with the somite is not so distinct. The lateral mesoderm splits some distance out into a thick somatic layer (*som.*) and a thin splanchnic layer (*spl.*). The longitudinal strand of cells (*le.*) immediately external to the edge of the medullary plate and passing inwards below it is also visible. (× 70.)

Fig. 9.—Transverse section through the 7th somite. In this section the Anlage of the Wolffian duct (*w. d.*) is seen to be distinct from the Anlage of the tubule (*w. t.*) underlying it. The latter is

more or less distinct both from the somite and the lateral mesoderm. The lateral mesoderm is not split, the ventral cœlom being absent in this region. The other relations are the same as in fig. 8. (× 70.)

Fig. 10.—Transverse section passing through both the 16th and 17th somites owing to their oblique direction. The somites in this region no longer possess distinct cavities—they consist of an upper and lower layer connected with each other by processes of the cells. Between the somites and the lateral mesoderm there is present the Anlagen of the Wolffian tubules in the form of a strand of cells (*w. t.*) slightly projecting from the intermediate cell mass. The cœlom (*cœ.*) is of great lateral extent, and numerous endothelial vessels (*b. v.*) are visible in the outer part of the section. (× 70.)

Fig. 11.—Transverse section through the blastopore (*bl.*) which is situated to one side of a longitudinal eminence at the anterior end of the primitive groove (*cf.* also fig. 23). The mesoderm is unsplit for a considerable distance out. (× 70.)

Fig. 12.—Transverse section through the primitive groove (*pr. g.*) slightly behind fig. 11. (× 70.)

Fig. 13.—Transverse section through the hinder region of the primitive streak (*pr. s.*). (× 70.)

Fig. 14.—Median portion of fig. 2 more highly magnified. The section passes through the anterior end of the notochord where it spreads out and is represented by an axial thickening of the entoderm. With this thickening the mesoblast is continuous laterally. (× 280.)

Fig. 15.—Longitudinal section of anterior end of embryo passing through the optic groove (*op. gr.*) and the head fold. (× 180.)

Fig. 16.—Longitudinal section of anterior region of the embryo, passing to one side of the median line, especially to show the mid-brain neuromere (*N.*) and the four hind-brain neuromeres ($n^{\prime}$-$n^{iv.}$). (× 70.)

Plate XII.

Fig. 17.—Transverse section through the anterior portion of the 4th neuromere showing the mesial and lateral bulgings on its under side—the lateral one much the larger of the two and projecting outwards. (× 180.)

Fig. 18.—Longitudinal section through the 3rd and parts of the 2nd and 4th neuromeres especially to show their arc-shaped form. (× 280.)

Fig. 19 (1-9).—Series of transverse sections through the neuromeric region of the hind-brain. Sections 2, 4, 6 and 8 pass through the neuromeres (n^{I}-n^{iv}), while 1, 3, 5, 7 and 9 show the normal thickness of the medullary plate in front of and between the neuromeres. (× 70.)

Fig. 20.—Transverse section through the anterior portion of the head process of the primitive streak. Three sections in front of the internal opening of the blastoporic canal. The lumina (*nch. c.*) in the head process represent the last traces of the chorda-canal. (× 340.)

Fig. 21.—Transverse section through the internal opening of the blastoporic canal (*bl. op*) into the cavity of the vesicle. (× 340.)

Fig. 22.—Transverse section through the head process of the primitive streak (*h. pr.*) 9 sections behind fig. 21. The blastoporic canal is represented by three lumina (*bl. c.*). The head process (*h. pr.*) is distinct from the ectoderm, but shows traces of cellular connection with the entoderm. It is more or less continuous with the mesoderm laterally. (× 340.)

Fig. 23.—Transverse section through the blastopore (*bl.*). It opens to one side of a longitudinal projection at the front end of the primitive groove Ectoderm, mesoderm and entoderm are fused with each other in the axial line. (× 340.)

Plate XIII.

Fig. 24.—Transverse section through the anterior end of the primitive groove (*pr. g.*) immediately behind fig. 24. As in that fig. the three germinal layers are continuous with each other axially. (× 340.)

Fig. 25.—Transverse section through the united Anlagen of the Wolffian duct and tubule in the region of the 7th somite. The Wolffian duct Anlage (*w. d.*) is semilunar in form and is connected at the middle of its ventral side with the tubule Anlage (*w. t.*). The latter possesses a small lumen and is distinct from the somite (*m. s.*), but connected with the lateral mesoderm (*mes. l.*). (× 340.)

Fig. 26.—Transverse section through the Anlagen of the Wolffian duct and tubule some distance behind fig. 25. The Wolffian duct Anlage (*w. d.*) now exists as a flattened band overlying and distinct from the rounded tubule Anlage. Its outer edges are closely approximated to the ectoderm which immediately over t

Anlage of the duct is very delicate, and, as in the preceding figure, has been broken in the process of section cutting. The tubule Anlage is rounded in shape, and consists of radiating cells surrounding a small lumen. It is now free from both the somite and the lateral mesoderm. (× 340.)

Figs. 27, 28 and 29 represent three successive sections through the posterior end of the Anlage of the Wolffian duct. As compared with fig. 26, the Wolffian duct Anlage in fig. 27 is now considerably smaller and thinner, and is connected at its outer edges with the ectoderm. In fig. 28 the Anlage is still more reduced in size, consisting of a single layer of cells, while in fig. 29 it is reduced to a single cell, connected at both ends with the ectoderm. Behind the section from which fig. 29 is drawn there is no longer any trace of the Wolffian duct Anlage. The Anlage of the tubule in fig. 27 appears as a rounded projection of the intermediate cell mass, with radially arranged cells, but in figs. 28 and 29 this radial arrangement is lost, and the Anlage appears as a slight elevation of the mass. (× 340.)

Fig. 30.—Transverse section through the wall of the blastodermic vesicle some distance beyond the amniotic area, showing the ectoderm (*ect.*), vitelline entoderm (*vit. ent.*) and mesoderm. The latter consists of a layer of cells below which occur numerous vasifactive cells (*vas. c.*) (× 320.)

Fig. 31.—Transverse section of the wall of blastodermic vesicle some distance further out than fig. 30. The mesoderm here consists of a single layer of spindle-shaped cells, while vasifactive cells have almost entirely disappeared. (× 320.)

Fig. 32.—Transverse section of wall of blastodermic vesicle at the antembryonic pole. Ectoderm and vitelline entoderm are alone present. (× 200.)

Fig. 33.—Vitelline entoderm cells of blastodermic vesicle drawn as seen through the ectoderm. They are filled up almost entirely by yolk spheres; their large and somewhat irregular nuclei are generally situated on the outer sides of the cells next the ectoderm. (× 200.)